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**Toxicological availability of nickel to the benthic oligochaete *Lumbriculus*  
*variegatus***

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## Abstract

It is generally accepted that the bioavailability of metals in sediments is influenced by the presence of acid volatile sulfides (AVS). The pore water hypothesis predicts that, if the molar concentration of simultaneously extracted metals (SEM) in a sediment is smaller than the molar concentration of AVS, the free metal ion activity in the pore water is very small and that consequently no metal toxicity in short-term toxicity tests is observed. In this study we examined (1) if this concept can be extended to predict the absence of chronic Ni toxicity to the oligochaete deposit-feeding worm *Lumbriculus variegatus* and (2) if the organic carbon normalized excess SEM; i.e.  $[\text{SEM-AVS}]/f_{\text{OC}}$  predicts the magnitude of Ni toxicity to *L. variegatus*. A 28-day toxicity experiment was performed in which biomass production of *L. variegatus* was determined in two natural sediments with different [AVS] and  $f_{\text{OC}}$ , spiked at different Ni concentrations. The absence of toxicity is predicted correctly by the  $[\text{SEM-AVS}] < 0$  criterion when only the 0-1 cm surface layer of the sediment is considered, but not when the whole bulk sediment is considered (0-3 cm). In both sediments, the same  $[\text{SEM-AVS}]/f_{\text{OC}}$  at the surface corresponds with a similar decrease in *L. variegatus* biomass. Thus,  $[\text{SEM-AVS}]/f_{\text{OC}}$  in the surface layer accurately predicts the magnitude of toxicity. This measure is therefore a good estimator of toxicologically available Ni. On the other hand, the free  $\text{Ni}^{2+}$  ion activity in the overlying water appeared to be an equally good predictor of the magnitude of toxicity. Consequently, it was not possible to determine the relative importance of the overlying water and pore water exposure route with the semi-static laboratory experiments.

## Introduction

It has been demonstrated that the simultaneously extracted metals – acid volatile sulfide (SEM-AVS) method is an effective tool in predicting the absence of metal toxicity in sediments in short-term toxicity tests when  $[\text{SEM-AVS}] < 0$  (Di Toro et al., 1990; Di Toro et al., 1992; Casas and Crecelius, 1994; Pesch et al., 1995; Hansen et al., 1996).

The underlying principle is that except for pyrite, all other iron and manganese mono sulfides that may be present in sediments have higher solubility products than other metal sulfides. Thus, Fe and Mn can be displaced by other divalent metals (Cu, Cd, Ni, Pb, Zn) on a mole-to-mole basis. Because these metal sulfides exhibit very low solubility, sediments with an excess of reactive sulfide will exhibit very low dissolved metal concentrations in pore waters and will not cause toxicity (Ankley et al., 1996).

Sediments with excess SEM do not always exhibit toxicity to invertebrate sediment organisms. Organic matter can bind non-sulfide bound trace metals, thus preventing them to enter the dissolved phase (Mahony et al., 1996). Based on this,  $[\text{SEM-AVS}]/f_{\text{OC}}$  has been proposed as a measure of bioavailable metal (Di Toro et al., 2005; Hansen et al., 2005). In a recently proposed biotic ligand model for sediments, it has been shown that  $[\text{SEM-AVS}]/f_{\text{OC}}$  determines the free metal activity in the pore water (Di Toro et al., 2005). If this value is greater than a critical threshold, sediments are predicted to be toxic.

The  $[\text{SEM-AVS}]/f_{\text{OC}}$  concept assumes that there is no metal toxicity caused by transformations of the sulfide and organic matter bound metal in the gut of sediment-ingesting organisms or via exposure to contaminated food (Meyer et al., 2005). Some authors (Lee B.G., Griscom S.B. et al., 2000; Lee B.G., Lee J.S. et al., 2000) have

observed that benthic organisms can assimilate metals that are associated with sulfides via dietborne exposure. They demonstrated significant bioaccumulation of metals at concentrations where  $[\text{SEM-AVS}] < 0$ . However, these authors did not measure toxic effects; they assume that dietary exposure as measured by accumulation is related to chronic toxicity (Lee B.G., Griscom S.B. et al., 2000). However, they also note that generic relationships between metal bioaccumulation and toxicity are not well understood. Animal species differ in their abilities to detoxify bioaccumulated metals (e.g. via metallothionein or granule induction) or develop tolerance (Adams et al., 2000; Lee B.G., Lee J.S. et al., 2000). An increase in bioaccumulation may be a sign of active metal uptake and not toxicity in the case of metal deficiency (Muyssen and Janssen, 2002; Bossuyt and Janssen, 2003). Thus, bioaccumulated metals may not always reflect toxic effects (McGeer et al., 2003).

To our knowledge, the applicability of the SEM-AVS concept to predict the absence of chronic, sub-lethal toxicity of nickel to a benthic organism exposed in single-species laboratory toxicity tests has not been demonstrated. In this study, the hypothesis tested is that  $\text{Ni}^{2+}$  in the pore water determines chronic toxicity to *L. variegatus*. In other words, we examined (1) if  $[\text{SEM-AVS}] < 0$  predicts the absence of chronic Ni toxicity and (2) if  $[\text{SEM-AVS}]/f_{\text{OC}}$  predicts the presence and magnitude of Ni toxicity to *L. variegatus*, independent of sediment characteristics. The vertical profiles of  $[\text{SEM-AVS}]$  and pore water concentrations were considered, since vertical distributions of AVS and SEM can affect metal toxicity in sediments and pore water metal concentrations (Boothman et al., 2001). Two natural sediments with different characteristics were chosen to test the hypothesis, so that at comparable  $[\text{SEM}]$ , different  $[\text{AVS}]$  accounted for differences in  $[\text{SEM-AVS}]$ . Finally, the applicability of

the mentioned sediment-biotic ligand model (sBLM) (Di Toro et al., 2005) will be tested with the data on biomass of *Lumbriculus* in Ni spiked sediments. In this way, the possible use of the sBLM for chronic single-species toxicity tests with Ni will be evaluated.

## **Materials and Methods**

### **Sediment sampling, spiking and analysis**

Two natural sediments were sampled. Brakel sediments were taken by scoop sampling from the banks of a small stream (near the source) in a nature reserve in Brakel, Belgium (50°45' N, 3°46' E). Water depth at the sampling time was about 50 cm. Ijzer sediments were taken by grab sampling with a Van Veen grab from the Ijzer river, Belgium (about 50°58'N, 2°48'E). This river is situated in a low density farming zone and has a water depth of about one meter. Plastic buckets were filled with 50-70% sediment, after which overlying water was added to the top of the bucket, which was subsequently closed with a plastic lid. After sampling, sediments were frozen at -20 °C for two days to kill indigenous organisms and subsequently stored at 4 °C until use. Brakel and Ijzer have a moderate and a high AVS content, respectively, and a low to moderate organic carbon content. Sediment characteristics are provided in Table 1.

Before spiking, the sediments were cleaned by press sieving with a 0.5 cm sieve in deaerated ( $<0.2$  mg O<sub>2</sub>/L) overlying water from the site of origin. Cleaned sediments were stored at 4°C for 48h sedimentation, after which the overlying water was carefully poured-off and spiking was started. Ni was added as a NiCl<sub>2</sub>-solution (Merck) in

deaerated ( $< 0.2$  mg/L  $O_2$ ) deionised water, which was thoroughly manually mixed with the sediments in airtight sealed plastic bags. Mean measured test concentrations of Ni in the spiked sediments ranged from 127 to 1458 mg/kg dry sediment (Brakel) and from 514 to 3847 mg/kg dry sediment (Ijzer) (Table 2). After spiking with Ni, sediments were placed into glass test vessels and were stabilized/equilibrated for 70 days before test initiation (Simpson et al., 2004). According to Simpson et al. (2004), equilibration of Ni-spiked sediments occurs within 30-70 days. In natural sediments, AVS consists largely of iron sulfides (Lee J.S. et al., 2000). When the spiked  $Ni^{2+}$  binds with AVS,  $Fe^{2+}$  is released from the Fe(II)S phase. Fe(II) can be lost by diffusion to the oxic layers, where it can be oxidized to solid Fe-hydroxide precipitates. After 43 days for the Brakel sediments and after 58 days for the Ijzer sediments, precipitated Fe(OOH) was removed by manually scraping it off the top layer. This was done to prevent toxicity due to elevated dissolved Fe in the pore water and overlying water (Gonzalez, 1996) and to prevent artefacts during the determination of SEM and AVS (Simpson et al., 1998).

At test initiation and termination, samples were taken for determination of dry wt, %OC, total Ni concentration ( $Ni_T$ ), AVS and  $SEM_{Ni}$ . Overlying water was sampled about 1 cm above the sediment surface to assess dissolved Ni, pH, ammonia, hardness, conductivity and dissolved organic carbon (DOC) (Table 3). Pore water was collected at each cm depth by means of inert passive pore water samplers with polyether sulfon membranes, so called mini-peepers (Doig and Liber, 2000). In each chamber, dissolved Ni, DOC, pH and redox potential were measured. Sediment dry weight is defined as the difference between wet and dry sediment (dried 72h at  $60^\circ C$ ). Organic carbon content was determined by loss on ignition (Egeler et al., 2005). AVS and  $SEM_{Ni}$  were determined according to the modified diffusion method (Leonard et al., 1996) (5 g

sediment extracted for 1 hour in 60 mL of 1N HCl). Samples were taken with a core (2 cm diameter) and divided into 1 cm sediment layers. Cores were taken from a replicate test vessel with overlying water present and subsections of 1 cm were immediately (within 5 seconds) inserted into the diffusion systems, to prevent oxidation and loss of AVS. Sediment destruction for total metal content was done by acid microwave digestion. Ni was analyzed using flame AAS (Spectra AA 100-Varian) and/or a graphite furnace AAS (Zeeman, SpectrAA300-Varian). The detection limit of the former is 8.7 µg Ni/L. For the lower nickel concentrations, the furnace was used with a detection limit which ranged between 2 and 3.4 µg Ni/L.

#### Test designs

*Lumbriculus variegatus* is an ecologically relevant oligochaete, occurring throughout Europe and the United States (Spencer, 1980). It is an epibenthic/benthic organism subject to contaminant exposure via all routes of concern, including ingestion of sediment particles (Phipps et al., 1993). The test was based on a proposed draft OECD test guideline (Egeler et al., 2005). Organisms were from an in-house culture, with parental organisms provided by Blades Biological LTD (United Kingdom). Biomass per replicate of ten organisms was evaluated as a chronic endpoint integrating growth and survival. *L. variegatus* reproduces parthenogenetically by fragmentation. Therefore survival *sensu stricto* cannot be measured by counting the number of organisms at the end of the test. Overlying water was a medium hard reconstituted water composed of the following salts diluted in deionised water: 4 mg/L KCl, 123 mg/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 96 mg/L NaHCO<sub>3</sub> and 6 g/L CaSO<sub>4</sub> (USEPA, 1985). Sixty to 70 % of the overlying water was renewed twice a week. The temperature was 23± 2 °C and a 16:8 hours light:dark



regime was applied. Ten adult organisms with complete regeneration of tail or head were added per replicate jar with 400 g wet sediment and 250 mL overlying water. Organisms were fed ground Tetramin<sup>TM</sup> fish flakes (200 µg per organism per day). Five replicates per concentration were used for biological endpoints, whereas two replicates were used to determine physico-chemical sediment properties. Tests were terminated after 28 days.

## Data treatment

Concentration - effect curves and EC<sub>50</sub>s were obtained using the logistic model. Parameter estimates were found by fitting the model to the observed data with a non-linear least squares estimation according to the Levenberg-Marquardt algorithm (Levenberg, 1944; Marquardt, 1963). Lowest observed effect concentrations (LOECs) were based on significant differences ( $p < 0.05$ ) of mean biomass between Ni treatments and control treatments, determined using the Mann-Whitney U test, adjusted for ties. All calculations are based on measured Ni concentrations. T-tests were performed on the residuals of fitted data for comparison of relationships between biomass and concentration after checking for normality with Kolmogorov-Smirnov goodness-of-fit test and for homogeneity of variances with Levene's test. All significance levels were set at  $p = 0.05$ . Statistics were performed using Statistica 6.0 software (Statsoft, Tulsa, OK, USA). Speciation calculations were performed with the Windermere Humic Aqueous Model (WHAM) VI version 6.0.8 (Natural Environment Research Council, UK) (Tipping, 1998) or with the Biotic Ligand Model (BLM) version 2.1.2 (Hydroqual, Mahwah, New Jersey), which incorporates WHAM V and with which BLM calculations were performed.

## Results and discussion

In comparison with AVS concentrations in different natural sediments reported in literature (van den Hoop et al., 1997; van den Berg et al., 1998; van den Berg et al., 2001), the sediments tested in this study can be regarded as having a medium and high AVS content. The increase in [AVS] at  $t_0$  from the top cm to the 2-3 cm sediment section, i.e. from 3.5  $\mu\text{mol/g}$  dry sediment to 13.5  $\mu\text{mol/g}$  dry sediment in Brakel and from 42.1  $\mu\text{mol/g}$  dry sediment to 118.2  $\mu\text{mol/g}$  dry sediment in IJzer, can be considered as representative for the AVS stratification observed *in situ* (van den Berg et al., 1998; van den Berg et al., 2001).

The LOECs expressed as [SEM-AVS] in the different sediment layers are given in Table 4. In the bulk (whole) core of the IJzer sediment [SEM-AVS]<sub>LOEC,bulk</sub> is  $< 0$ . This means that a significant toxic effect was observed, although the concept predicts that no toxicity is expected at [SEM-AVS]  $< 0$ . [SEM-AVS]  $< 0$  measured on the bulk sediment basis does not predict the absence of chronic toxicity. However, when the surface layer of the sediments is considered (0-1 cm), [SEM-AVS]<sub>LOEC,surface</sub>  $> 0$ , confirming the concept. Following the SEM-AVS concept, this may suggest that the surface layer of the sediment contributes more to the observed toxicity than the deeper sediment layers when [SEM-AVS]  $< 0$  in the deeper layers. Due to oxidation of AVS, more non-sulfide bound Ni will be present at the surface, resulting in the positive [SEM-AVS] values, as observed for other metals by other authors (DeWitt et al., 1996; Liber et al., 1996). Negative values for [SEM-AVS]<sub>surface</sub> were only found at lower Ni concentrations in both sediments, where no significant toxic effect was observed. The use of the SEM-AVS concept for risk assessment procedures or for determining sediment quality criteria

should preferentially consider surface layer-based analyses as an alternative to bulk sample analyses (e.g. 10 cm, (van den Hoop et al., 1997)). Obviously, the potential contribution to toxicity from nickel in the overlying water should not be disregarded either in risk assessment (see also further).

In this study, no effect was observed at  $[\text{SEM-AVS}]_{\text{surface}} < 0$ . Lee et al. (Lee B.G., Griscom S.B. et al., 2000; Lee B.G., Lee J.S. et al., 2000) did observe accumulation of metals in clams and marine polychaetes at  $[\text{SEM-AVS}] < 0$ , explaining this by dietary metal uptake from ingested sediments being the dominant exposure route. These authors assumed, but did not demonstrate, that dietary exposure as measured by bioaccumulation is at least generally related to chronic toxicity (Lee B.G., Griscom S.B. et al., 2000; Lee B.G., Lee J.S. et al., 2000). Possibly, bioaccumulation from the dietary route at  $[\text{SEM-AVS}] < 0$  in our study was small enough not to cause significant toxicity.

To investigate the utility of  $[\text{SEM-AVS}]/f_{\text{OC}}$  as a measure of toxicologically available Ni, the biomass data of both Brakel and Ijzer sediments were pooled and their relationship with  $[\text{SEM-AVS}]/f_{\text{OC}}$  was analyzed. Negative values of  $[\text{SEM-AVS}]/f_{\text{OC}}$  were omitted, following the concept that no toxicity occurs when  $[\text{SEM-AVS}] < 0$ , as demonstrated with the positive  $[\text{SEM-AVS}]_{\text{LOEC, surface}}$ . The effect on biomass of *L. variegatus* as a function of  $[\text{SEM-AVS}]/f_{\text{OC}}$  in the surface layer,  $[\text{SEM-AVS}]/f_{\text{OC, surface}}$ , showed a similar trend in Brakel and Ijzer sediments. Using the positive values of  $[\text{SEM-AVS}]/f_{\text{OC}}$  in the surface layer (0–1 cm) of the sediments, one concentration-effect curve can be fit to the pooled data of both sediments (Fig. 1a), with the mean of the residuals of both sediments not significantly differing (t-test,  $p = 0.14$ ). This indicates that  $[\text{SEM-AVS}]/f_{\text{OC}}$ , or the equivalent ( $\text{Ni}^{2+}$ ) in the pore water, is indeed a good

measure for the toxicologically available Ni. However, using the positive values of [SEM-AVS]/ $f_{OC}$  in the deeper layers (1-2 cm and 2-3 cm), also one concentration-effect curve can be fit to the pooled data of both sediments, although only one positive value of [SEM-AVS]/ $f_{OC}$  remains for Ijzer (data not shown). This raises the question whether all layers contribute equally to Ni toxicity when there is a positive [SEM-AVS]/ $f_{OC}$ . If this is the case, the organisms would be exposed to an average [SEM-AVS]/ $f_{OC}$  over the entire sediment depth. Those averages were calculated for both sediments, with negative values set to zero, following the concept that this cannot elicit a toxic effect. One concentration-effect curve can be fitted to the pooled data (Fig. 1b), also with the means of the residuals for both sediments not significantly differing (t-test,  $p = 0.89$ ). The fit is similar ( $R^2 = 0.453$ ) to the fit in Fig. 1a in which surface layer data only are represented ( $R^2 = 0.402$ ), indicating that *L. variegatus* may also be exposed to the deeper layers with lower [SEM-AVS]/ $f_{OC}$ . During the test, the organisms were observed to be mainly burrowed in the sediment, down to varying depths of maximum 3 cm.

The difference between the  $EC_{50}$ s for biomass in both sediments is reduced from a factor 2.9 when expressed as total Ni in the bulk sediment ( $\mu\text{g/g}$  dry sediment) to a factor 1.6 when expressed as [SEM-AVS]/ $f_{OC}$  in the surface layer. According to a t-test comparing the  $EC_{50}$ s in both sediments, the factor 2.9 difference is highly significant ( $p = 0.00001$ ), while the factor 1.6 difference is not significant ( $p = 0.51$ ). Thus, the same intensity of toxic effect (50% reduction of growth) is observed at not significantly differing concentrations of [SEM-AVS]/ $f_{OC}$  in two different sediments. This suggests again that toxicity to *L. variegatus* relates to [SEM-AVS]/ $f_{OC}$  or free  $\text{Ni}^{2+}$  in the pore water. These results support the use of [SEM-AVS]/ $f_{OC}$  as a predictor of sediment toxicity.

The concentration of  $[\text{SEM-AVS}]/f_{\text{OC}}$  determines the free  $[\text{Ni}^{2+}]$  in the pore water (Di Toro et al., 2005). Using the dissolved  $[\text{Ni}]$  in the pore water of the surface layer of the sediments, one concentration-response curve can be fitted to the pooled data of the Brakel and Ijzer sediments (Fig. 1c). However, this fit was not as good as the one obtained with  $[\text{SEM-AVS}]/f_{\text{OC}}$ : there was a significant difference between the residuals of both sediments (t-test,  $p = 0.02$ ). This may be explained by different ratios of dissolved  $[\text{Ni}]$  and free  $(\text{Ni}^{2+})$  in the pore water in both sediments, due to different pore water characteristics.

According to the SEM-AVS concept, due to the small solubility product constants of metal sulfides, sediments with an excess of AVS are expected to have very low metal activity in the pore water (Berry et al., 1996). However, in this study rather high Ni concentrations were found in the pore water of sediment layers in which  $[\text{SEM} - \text{AVS}]$  was smaller than zero. For instance, in the 2-3 cm horizon of Ijzer,  $[\text{SEM-AVS}] = -14.5 \mu\text{mol/g}$ , while  $[\text{Ni}]_{\text{PW}} = 1221 \mu\text{g/L}$ . This is consistent with Gonzalez (1996) and Doig and Liber (2006), who also observed high  $[\text{Ni}]_{\text{PW}}$  at SEM/AVS ratios smaller than one. One possible explanation could be that small colloidal NiS particles passed through the  $0.45 \mu\text{m}$  peeper membrane and were measured as “dissolved” Ni in the pore water (Leonard et al., 1999). Another possibility could be that nickel polysulfide complexes or nickel bisulfide ( $\text{NiHS}^+$ ) complexes, which are soluble and do not react with solid FeS to form insoluble NiS, were present (Doig and Liber, 2006). A third possibility would be that the system is not in equilibrium yet, even after the 70 days equilibration period as recommended by Simpson et al. (2004). This last option seems less likely because of the visible FeOOH precipitate that formed during the equilibration time as described

above, indicating the replacement of Fe by Ni in the FeS. This precipitate did not form again after it was removed, suggesting that the formation of NiS has ended and equilibrium is achieved.

The recently proposed sediment-biotic ligand model (sBLM) (Di Toro et al., 2005) predicts the toxicity of sediments based on  $[\text{SEM-AVS}]/f_{\text{OC}}$ , assuming that organic carbon and AVS are the only relevant metal-partitioning phases in sediments. The LOECs expressed as  $[\text{SEM-AVS}]/f_{\text{OC, surface}}$  are 122  $\mu\text{mol/g OC}$  and 495  $\mu\text{mol/g OC}$  for Brakel and Ijzer, respectively. These results corroborate the observation that the onset of toxicity occurs at  $[\text{SEM-AVS}]/f_{\text{OC}} \approx 100 \mu\text{mol/g OC}$ . This is an empirical observation by Di Toro et al. (2005) based on a series of sediment toxicity data from acute tests with different metals, mainly with marine amphipods and from chronic tests with Cd and Zn, where effect/no effect was considered as a measure of toxicity in colonization or long term single-species studies. This observation was also made by Burton et al. (2005) for Zn-toxicity in colonization studies with sediments in the field.

Specifically for Ni, Di Toro et al. (2005) have, with the sBLM, calculated critical values of  $[\text{SEM-AVS}]/f_{\text{OC}}$  for a range of water types with varying pH, based on the acute *Daphnia* critical gill-Ni accumulation. The chronic LOECs in our study, where the average  $\text{pH}_{(\text{surface layer})}$  was 7.6 and 7.1 for Brakel and Ijzer respectively, are lower than the critical acute Ni concentrations ( $\text{LC}_{50}$ ) of 642 - 1057  $\mu\text{mol/g OC}$ , calculated with the sBLM, in a standard freshwater at pH 7.0 – 8.0 (Di Toro et al., 2005). Thus, our results do not confirm the sBLM as opposed to the studies cited by Di Toro et al. (2005) in which no chronic effects are observed under the lowest calculated (acute) critical value of  $[\text{SEM-AVS}]/f_{\text{OC}}$ . This might be due to the fact that the *chronic* results from these

study with Ni and the freshwater organism *L. variegatus* are lower than the *acute* results on which Di Toro et al. based their critical values, and/or due to the fact that the *Daphnia magna* BLM is not applicable to *Lumbriculus variegatus* due to different sensitivities of both organisms. However, Lumbriculidae are generally less sensitive to metals than *Daphnia magna* (Von der Ohe and Liess, 2004), and as such one would expect the LOEC to be higher than the sBLM-predicted critical [SEM-AVS]/ $f_{OC}$ . A critical accumulation was calculated based on the 96 h LC50 for *L. variegatus* reported by Schubauer-Berigan et al. (1993). Using this new parameter and the average measured pore water chemistry, the sBLM calculated values of critical [SEM-AVS]/ $f_{OC}$  are 2060  $\mu\text{mol/g OC}$  and 1780  $\mu\text{mol/g OC}$  at a pH of 7.6 (Brakel) and 7.1 (Ijzer), respectively, which is indeed larger than the values based on *Daphnia magna* sensitivity (Di Toro et al., 2005). Knowing that the acute to chronic ratio for Ni is about 30 for *Daphnia magna* (Hunt et al., 2002), it is more likely that the difference between acute and chronic sensitivity is the major cause of the LOECs being lower than the sBLM-predicted critical [SEM-AVS]/ $f_{OC}$ . The sBLM as applied by Di Toro et al. (2005), does not work for our chronic toxicity data of *L. variegatus* biomass. The acute *Daphnia magna* BLM underestimates Ni toxicity in this case and further evaluation of the sBLM for chronic endpoints will be necessary. It is possible, however, that elevated overlying water concentrations at the LOECs in our experiments have a confounding effect on the interpretation of [SEM-AVS]/ $f_{OC}$ , as explained below. This can be another explanation for the observation of LOECs expressed as [SEM-AVS]/ $f_{OC}$  below model predicted critical values.

This discussion focused on the [SEM-AVS]/ $f_{OC}$  or  $\text{Ni}^{2+}$  in the pore water of the sediments as exposure route for Ni toxicity to *L. variegatus*. However, it should be

noted that the Ni concentrations in the overlying water of the tested sediments were elevated in the higher treatments, e.g. mean overlying water concentrations at the LOEC of 248 µg/L Ni for the Brakel treatment with 544 mg Ni/kg dry wt (mean) and 1135 µg/L Ni for the Ijzer treatment with 2234 mg Ni/kg dry wt (mean) (Table 2).  $\text{Ni}^{2+}$  activity in the overlying water,  $(\text{Ni}^{2+})_{\text{OW}}$ , was calculated with two methods. Firstly, speciation was calculated with WHAM VI, with  $\log K_{\text{MA}}(\text{Ni})=1.75$  and assuming that DOC consists of 40% active fulvic acid, as the latter was shown to yield a good fit between observed and measured free  $\text{Ni}^{2+}$  in natural surface waters (Van Laer et al., 2006). Secondly, speciation calculations were performed following Di Toro et al. (2005), using the BLM software, with the default  $\text{pK}_{\text{Ni-HA}} = 2.7$  and 1.4 for humic and fulvic acids respectively and assuming that DOC consists of 84% humic acid and 16% fulvic acid. To investigate the possibility of exposure to Ni via the overlying water, a concentration-effect curve was fitted to the pooled data of  $(\text{Ni}^{2+})_{\text{OW}}$  in the overlying water of Brakel and Ijzer sediments (Fig. 2). Only concentrations with a positive [SEM-AVS]/ $f_{\text{OC}}$ , i.e. concentrations where a significant effect was observed, were considered. This yields a good fit with no significant difference between the residuals of both sediments (t-test,  $p = 0.11$  and  $0.10$  for WHAM VI and BLM speciation respectively). The  $\text{EC}_{50}$  as  $(\text{Ni}^{2+})$  in the overlying water is  $9.6 \mu\text{mol/L}$  (95% confidence interval of  $6.5$  to  $14.1 \mu\text{mol/L}$ ) and  $4.2 \mu\text{mol/L}$  (95% confidence interval of  $2.3$  to  $7.7 \mu\text{mol/L}$ ) for the WHAM VI and BLM speciation calculations, respectively.

The Ni activity in the overlying water is an equally good predictor of toxicity as [SEM-AVS]/ $f_{\text{OC}}$ . Thus, it cannot be excluded that Ni in the overlying water is an important part of the toxicologically available Ni in our experiments.



The  $(\text{Ni}^{2+})_{\text{OW}}$  as calculated with WHAM VI correlated significantly with  $[\text{SEM-AVS}]/f_{\text{OC}}$  in the surface layer ( $R = 0.96$ ,  $p = 0.008$ ), but not significantly ( $R = 0.84$ ,  $p = 0.08$ ) with the averaged  $[\text{SEM-AVS}]/f_{\text{OC}}$  over depth as described above (with negative  $[\text{SEM-AVS}]/f_{\text{OC}}$  set to zero). Similar results were found for  $(\text{Ni}^{2+})_{\text{OW}}$  as calculated with the BLM. This is not surprising, since the Ni in the overlying water originates from a flux from the sediment via the pore water in the surface layer to the overlying water. So although the results of this study do not allow to distinguish  $(\text{Ni}^{2+})_{\text{OW}}$  from  $(\text{Ni}^{2+})_{\text{PW}}$  ( $\text{Ni}^{2+}$  activity in the pore water) as the exposure route of Ni for *L. variegatus*, it can be concluded that in these tests, either directly (via pore water) or indirectly (via overlying water),  $(\text{Ni}^{2+})_{\text{PW}}$  or the equivalent  $[\text{SEM-AVS}]/f_{\text{OC}}$  determines the toxicologically available Ni for *L. variegatus*.

In historically contaminated sediments in the field, pore water concentrations are likely to be higher than overlying water concentrations, due to higher dilution factors than those occurring in the routinely used laboratory test designs: i.e. static renewal. Also, the proportion of nickel in the solid phase will be higher in natural sediments, as nickel does not ordinarily enter sediments in soluble forms, as was the case with this study. Therefore  $[\text{SEM-AVS}]/f_{\text{OC}}$  is suggested as a measure of bioavailable Ni for risk assessment procedures, provided that overlying water  $(\text{Ni}^{2+})$  is measured and sufficiently low. More research is needed to establish the relative importance of pore water and overlying water as exposure routes for Ni to *L. variegatus*. Care should be taken in laboratory experiments with high Ni concentrations in sediment, if the purpose is to test the toxicity of nickel associated with sediment phases (i.e., particles and pore water) and not of the overlying water.

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544 Table 1 – Sediment characteristics of Brakel and Ijzer sediments: [AVS] in the control  
 545 treatments at  $t_0$  (test initiation) per horizontal layer under the surface, average  
 546 mass fraction of organic carbon in the tested sediments ( $f_{OC}$ ) and particle size  
 547 distribution.  
 548  
 549

Sediment	[AVS] (mmol/kg dry wt)			$f_{OC}$	% sand	% silt	% clay
	0-1 cm	1-2 cm	2-3 cm				
<b>Brakel</b>	3.5	9.9	13.5	0.0221	62.9	24.5	12.7
<b>Ijzer</b>	42.1	104.3	118.0	0.0395	23.9	46.4	29.6

550 Table 2: Measured concentrations of total Ni in the sediment, [Ni]<sub>ow</sub> (dissolved Ni in the overlying water), [SEM-AVS] and [Ni]<sub>PW</sub>  
551 (dissolved Ni in the pore water) of Brakel and Ijzer sediments, at the beginning (t<sub>0</sub>) and at the end (t<sub>end</sub>) of the 28 day test period, for  
552 the different tested concentrations, per horizontal layer when applicable and mean biomass per replicate of *L. variegatus* at the end  
553 of the test period, expressed as % of the mean biomass per replicate in the control treatments  
554  
555

### Brakel

Treat- ment	Total Ni (mg/kg dry wt)		[Ni] <sub>ow</sub> (µg/L)		[SEM-AVS] (µmol/g dry wt)						[Ni] <sub>PW</sub> (µg/L)						Biomass (% of control)
					0-1 cm		1-2 cm		2-3 cm		0-1 cm		1-2 cm		2-3 cm		
	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>end</sub>
control	8	5	7	3	-3.4	-1.5	-9.9	-5.9	-13.4	-10.3	11	32	12	17	28	17	100
C1	132	122	22	24	-2.2	-1.4	-8.3	-7.4	-12.2	-7.9	ND	128	ND	62	51	48	85.9
C2	201	179	21	35	-4.7	-3.4	-7.9	-8.8	-8.7	-5.7	ND	385	64	100	47	126	89.1
C3	547	540	263	233	1.8	3.6	0.9	0.9	0.9	1.0	351	1949	550	603	219	270	67.1
C4	1075	1068	941	967	12.6	14.1	7.1	10.8	9.4	9.6	1370	ND	1542	3343	3959	ND	39.9
C5	1544	1373	1986	2034	15.5	20.6	19.1	18.8	20.3	17.6	23000	8122	24177	7740	37474	10532	37.9

### Ijzer

Treat- ment	Total Ni (mg/kg dry wt)		[Ni] <sub>ow</sub> (µg/L)		[SEM-AVS] (µmol/g dry wt)						[Ni] <sub>PW</sub> (µg/L)						Biomass (% of control)
					0-1 cm		1-2 cm		2-3 cm		0-1 cm		1-2 cm		2-3 cm		
	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>0</sub>	t <sub>end</sub>	t <sub>end</sub>
control	8	16	13	19	-42.0	-24.0	-104.2	-86.6	-118.1	-87.6	6	7	16	11	13	10	100
C1	460	567	36	46	-39.4	-22.7	-109.7	-66.9	-111.6	-83.0	664	205	62	254	94	63	105.9
C2	759	905	117	88	-47.1	-37.0	-109.2	-77.8	-104.2	-75.8	924	ND	146	ND	57	205	88.4
C3	1486	1484	346	209	-13.5	3.8	-51.5	-49.6	-60.2	-48.4	985	695	1441	608	2284	499	72.1
C4	2271	2197	1141	1128	11.9	27.1	-14.1	-16.0	-14.1	-14.5	3492	2171	4649	1124	4689	1221	54.1
C5	3664	4031	2734	2383	31.5	49.5	9.1	27.3	20.7	23.4	10718	3412	ND	4963	ND	4847	41.8

556



557 Table 3 – Overlying water characteristics per treatment for the Brakel and Ijzer sediment tests: pH, dissolved Ca and Mg and DOC, at the  
558 beginning ( $t_0$ ) and at the end ( $t_{\text{end}}$ ) of the 28 day test period.  
559

<b>Brakel</b>								
<b>Treatment</b>	<b>pH</b>		<b>[Ca] (mg/L)</b>		<b>[Mg] (mg/L)</b>		<b>[DOC] (mg/L)</b>	
	$t_0$	$t_{\text{end}}$	$t_0$	$t_{\text{end}}$	$t_0$	$t_{\text{end}}$	$t_0$	$t_{\text{end}}$
control	8.03	7.90	36.58	39.06	11.88	14.02	1.318	1.670
C1	7.94	7.82	33.53	32.96	9.86	13.01	1.067	4.844
C2	8.17	7.83	33.80	34.64	10.11	12.89	1.173	1.884
C3	7.82	7.65	27.88	26.65	8.21	11.58	1.013	2.012
C4	7.87	7.57	29.11	25.51	8.37	11.28	1.118	2.752
C5	7.69	7.51	30.06	24.66	8.35	11.15	1.369	1.726

<b>Ijzer</b>								
<b>Treatment</b>	<b>pH</b>		<b>[Ca] (mg/L)</b>		<b>[Mg] (mg/L)</b>		<b>[DOC] (mg/L)</b>	
	$t_0$	$t_{\text{end}}$	$t_0$	$t_{\text{end}}$	$t_0$	$t_{\text{end}}$	$t_0$	$t_{\text{end}}$
control	7.70	7.99	36.29	48.29	15.952	12.877	1.067	2.403
C1	7.89	8.03	35.95	41.59	15.23	11.776	1.228	2.032
C2	7.63	7.77	30.85	23.13	12.631	7.899	1.226	2.547
C3	7.40	7.63	32.84	25.26	13.91	8.343	1.539	2.471
C4	7.43	7.42	33.33	26.33	13.586	8.37	1.345	1.934
C5	7.40	7.37	37.70	25.26	13.479	7.965	1.424	2.23

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562 Table 4 - LOECs for biomass/replicate of *L. variegatus* in Brakel and Ijzer sediments expressed as [SEM-AVS] ( $\mu\text{mol Ni/g}$  dry sediment)  
563 at different depths below the sediment surface at the beginning ( $t_0$ ) and at the end ( $t_{\text{end}}$ ) of the test, as well as the mean of the [SEM-  
564 AVS]<sub>LOEC</sub> at  $t_0$  and  $t_{\text{end}}$   
565

	Brakel			Ijzer		
	LOEC as [SEM-AVS] at $t_0$	LOEC as [SEM-AVS] at $t_{\text{end}}$	mean LOEC as [SEM-AVS]	LOEC as [SEM-AVS] at $t_0$	LOEC as [SEM-AVS] at $t_{\text{end}}$	mean LOEC as [SEM-AVS]
<b>Total core, 0-3 cm</b>	-0.4	1.5	0.6	-9.6	-7.4	-8.5
<b>A-horizon, 0-1 cm</b>	1.8	3.7	2.7	11.9	27.1	19.5
<b>B-horizon, 1-2 cm</b>	0.9	0.9	0.9	-14.1	-16.0	-15.0
<b>C-horizon, 2-3 cm</b>	0.9	1.0	0.9	-14.1	-14.5	-14.3

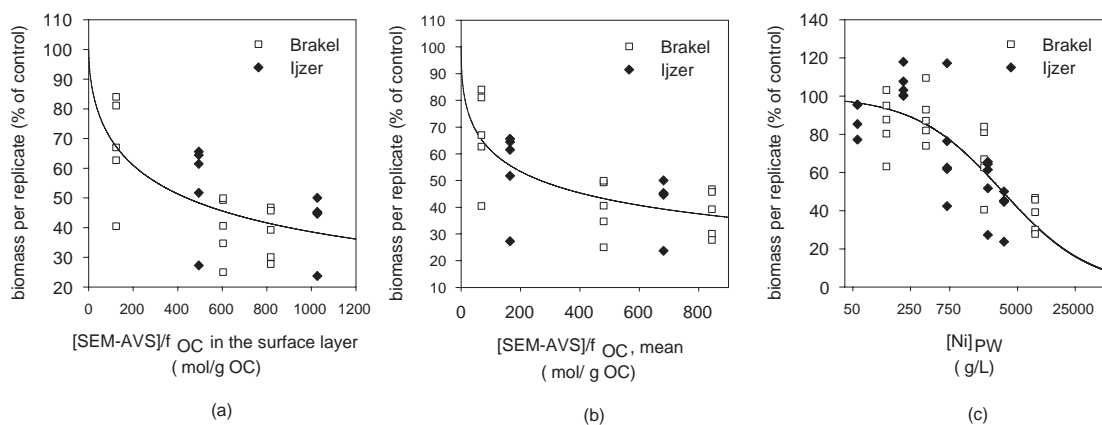
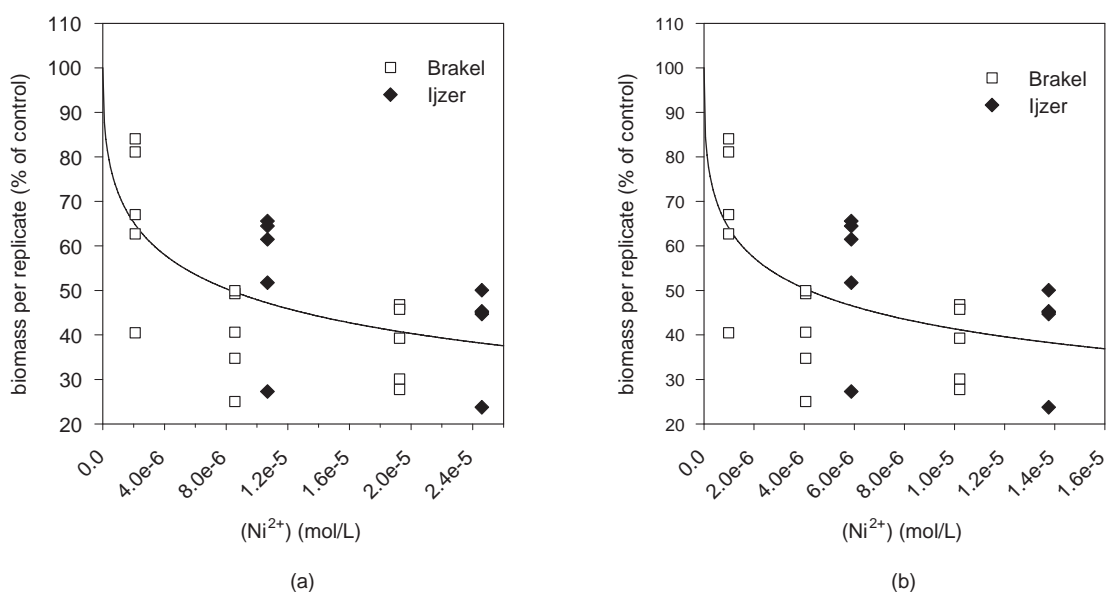


Figure 1: (a) Concentration – effect curve of biomass per replicate as a function of mean [SEM-AVS]/f<sub>OC</sub> in the surface layer of Brakel and Ijzer; (b) Concentration – effect curve of biomass per replicate as a function of mean [SEM-AVS]/f<sub>OC</sub> as mean of the three sediment layers (0-1, 1-2 and 2-3 cm depth) of Brakel and Ijzer; (c) Concentration - effect curve of biomass per replicate as a function of dissolved [Ni] in the pore water of the surface layer of Brakel and Ijzer at the end of the test

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Figure 2: (a) Concentration-effect curve of biomass per replicate as a function of the mean  $\text{Ni}^{2+}$  activity as calculated with WHAM VI (see text) in the overlying water of those concentrations with positive [SEM-AVS] of Brakel and Ijzer sediments; (b) Concentration-effect curve of biomass per replicate as a function of the mean  $\text{Ni}^{2+}$  activity as calculated with the BLM speciation programme (see text) in the overlying water of those concentrations with positive [SEM-AVS] of Brakel and Ijzer sediments.